Meat Curing
The Action of NaCl on Meat Electrolyte Binding

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SUMMARY

Blended mixtures of meat and water containing different concentrations of NaCl were stored for 24 hr at 3°C and further stored for 1 hr at either 3 or 70°C. Then water, nitrogen, chloride, pH, sodium, potassium, calcium, magnesium, and zinc were determined in aqueous extracts obtained by centrifugation. The results show that little or no sodium, potassium, or chloride was bound at 3 and 70°C. At 3°C, the addition of NaCl resulted in an increase of free calcium, magnesium, and, to a lesser extent, zinc. On heating at 70°C, with no added NaCl, all of the magnesium was free, soluble calcium increased, and zinc decreased. At 70°C, on addition of NaCl, free calcium increased and zinc decreased. Zinc was the only electrolyte that was substantially and strongly associated or bound with soluble protein.

INTRODUCTION

Cured meat and meat products in excess of 7 billion pounds are produced annually in this country. Improvements are constantly sought to improve both quality of products and efficiency of production. The changes in curing methods that have recently evolved have been aimed chiefly at reducing curing time and streamlining production methods. Further development of process modifications and improvements can be greatly aided by a more accurate and comprehensive knowledge of the mechanisms of curing.

The effects of added electrolytes have been stressed in previous investigations of curing. Also prominent among the subjects investigated have been color development and stability, penetration of curing agents, and, particularly, water retention. Evidence has also been obtained indicating that natural meat electrolytes, particularly Ca and Mg, have a role in determining the properties of cured meats (Wierbicki *et al.*, 1957a,b). This role is not clear, owing in part to a lack of agreement among investigators, but mainly to the fact that investigation has been relatively limited in amount. Existing information on the reorganization of natural meat electrolytes after the death of animals, during and after rigor (Hamm, 1958, 1959; Arnold *et al.*, 1956), and the interactions that follow the addition of curing agents, is valuable but not adequate in any of its phases.

The present investigation deals with determining the extent of the movement and binding of natural meat electrolytes following the addition of NaCl, the principal curing agent. In this, blended mixtures of meat and water containing increasing concentrations of NaCl were produced, stored 24 hr at 3°C, and then further stored 1 hr at either 3 or 70°C. Then water, nitrogen, chloride, pH, sodium, potassium, calcium, magnesium, and zinc were determined in centrifugally obtained aqueous extracts. The purpose was to determine whether electrolytes became redistributed between the soluble phases of blended meat and water mixtures at 3°C with the addition of NaCl, whether this distribution was affected by increasing concentrations of NaCl, and the changes that occur on heating at 70°C. To differentiate the quantities of free and protein-bound electrolytes in the aqueous phase, fractionation of free and protein-bound electrolytes was accomplished by ultracentrifugation.

EXPERIMENTAL

Preparation of samples. Rounds of eight steers were obtained 6–8 days after slaughter. Samples from the muscles of three were used in determining free electrolytes in aqueous phases as affected by treatments with NaCl and heat (Tables 1, 2, 3, 4; Figs. 1, and 2); those from two others were used in investigating protein sedimentation by ultracentrifugation (animals A and B, Table 5); and those from three were used in determining the amounts of ions bound by proteins (Tables 6, 7, and 8). The semimembranosus and semitendenosus muscles were removed and trimmed to remove separable fat and connective tissue, and portions were cut into cubes. The cubes were ground twice through an electric food chopper, and the ground meat was mixed thoroughly. Slurries were prepared by mixing meat 1:2 with ice-cold water (w/w) to which solid NaCl was added to produce concentrations of 0.97, 1.9, and 3.7% NaCl in the water present (added water plus the water content of the meat). The actual NaCl concentrations obtained as determined by measurements of sodium and chloride content varied slightly from the latter figures because of small differences in the moisture content of the meat. The slurries were then homogenized in a Servall Omnimixer, the cup being immersed in an ice bath. (Trade names are mentioned for identification, implying no endorsement.) The machine was operated for four 15-second intervals interspaced with 15-second cooling periods, which avoided noticeable temperature rise. The homogenates were then stored for 24 hr at 3°C.

Preparation of supernatant fractions. Aqueous extracts were prepared from portions of the homogenates after storage at 3°C, whereas others were prepared after heating the homogenates at 70°C. To prepare the former, portions of homogenates that had been stored at 3°C were centrifuged in a Servall centrifuge with head SS-1 for 60 minutes at 20,000 rpm (48,200 × G). Homogenates to be heated were transferred to centrifuge bottles and heated 1 hr at 70°C in a water bath. The heated homogenates were centrifuged with head SS-2 for 30 min at 10,000 rpm (16,300 × G), and the supernatant fraction recentrifuged with head SS-1 for 30 min at 20,000 rpm (48,200 × G). The supernatant fractions obtained were decanted and stored at 3°C for subsequent analysis and ultracentrifugation.

Preparation of protein-free supernatant fractions. Supernatant fractions from homogenates containing 0.0 and 3.7% added NaCl, representing both 3 and 70° treatments, were ultracentrifuged under refrigeration in a Spinco Model L fitted with head No. 40 for 24–32 hours at 40,000 rpm (144,000 × G). The top third portion of clear liquid was removed and stored at 3°C until analyzed.

Analytical determinations. Samples of meat and aliquots of supernatant fractions were ashed by a procedure described previously (Berman, 1960). Stock solutions were prepared from these ashed samples, and suitable aliquots were used for the determination of sodium, potassium, calcium, magnesium, and zinc. Sodium and potassium were determined by flame photometry, corrections being made for the presence of each other (Beckman Manual, 1954). Calcium was directly determined by EDTA titration (Patton and Reeder, 1956); magnesium was determined as the magnesium butyl amine hydroxyquinoline complex in chloroform (Umland and Hoffman, 1957); and zinc was determined as the dithizonate in carbon tetrachloride (Sandell, 1950). Chloride, total nitrogen, non-protein nitrogen, and water content and pH values were determined on separate aliquots of the extracts. Chloride was determined with a modified Volhard method (Caldwell and Moyer, 1935), and total nitrogen as described by the Official Methods of Analysis (AOAC, 1955). Moisture content of both residues and supernatant fractions was determined by the oven-drying method (Windham, 1953). Non-protein nitrogen was determined by the micro-Kjeldahl method on clear filtrates obtained after precipitation of the proteins with tungstic acid (Folin and Wu, 1919). A Beckman Model G pH meter was used to determine pH values. The ultracentrifugal sedimentation patterns were determined with a Spinco Model E ultracentrifuge at 20°C and 59,780 rpm.

RESULTS

Composition of meat samples. Table 1 shows the moisture, nitrogen, and electrolyte content of the meat samples.

pH values and nitrogen and water content. Table 2 shows the pH values and the nitrogen and water content of the supernatant fractions from the unheated and heated homogenates. At 3°C, increasing the concentration of NaCl decreased moisture content, increased nitrogen content, and had little effect on the pH values of the supernatant fractions. At 70°C, with increasing concentration of NaCl, moisture content decreased and nitrogen content increased. A small increase in pH was produced by addition of NaCl. Heating to 70°C increased pH and water content, and decreased the nitrogen content of the supernatant fractions.

Electrolyte content of supernatant fractions. Tables 3 and 4 show the electrolyte content of the extracts of unheated and heated homogenates. At 3°C, increasing concentrations of NaCl increased sodium, chloride, calcium, magnesium, and

Table 1. Average (3 animals) composition of meat samples (mg/g meat).

Moisture	Nitrogen	Cl	Na	K	Ca	Mg	Zn
739	34.85	0.380	0.394	4.10	.0323	0.218	.0267

Table 2. Average (3 animals) pH values, nitrogen, and water content of supernatant fractions of heated and unheated homogenates.

	pH				Water content				Nitrogen content			
	% NaCl				% NaCl				% NaCl			
Treatment	3.7	1.9	0.97	0.0	3.7	1.9	0.97	0.0	3.7	1.9	0.97	0.0
3°C	5.50	5.52	5.50	5.51	.930	.950	.960	.973	5.38	4.59	4.41	3.95
Heated at 70°C	5.75	5.76	5.79	5.70	.948	.964	.974	.984	1.99	1.90	1.85	1.80

Table 3. Average (3 animals) electrolyte content of the supernatant fractions of homogenates treated at 3°C.

Cl mg/g H_2O				Na mg/g H_2O				K mg/g H_2O			
% NaCl				% NaCl				% NaCl			
3.7	1.9	0.97	0.0	3.7	1.9	0.97	0.0	3.7	1.39	0.97	0.0
22.3	11.3	5.70	0.126	15.0	7.61	4.01	0.140	1.40	1.44	1.49	1.49

Ca (mg/g H_2O) $\times 10^3$				Mg (mg/g H_2O) $\times 10^3$				Zn (mg/g H_2O) $\times 10^3$			
% NaCl				% NaCl				% NaCl			
3.7	1.9	0.97	0.0	3.7	1.9	0.97	0.0	3.7	1.39	0.97	0.0
9.47	7.51	5.89	5.50	75.9	72.1	70.4	53.1	5.15	5.14	5.18	4.41

Table 4. Average (3 animals) electrolyte content of the supernatant fractions of homogenates heated at 70°C.

Cl mg/g H_2O				Na mg/g H_2O				K mg/g H_2O			
% NaCl				% NaCl				% NaCl			
3.7	1.9	0.97	0.0	3.7	1.9	0.97	0.0	3.7	1.9	0.97	0.0
22.2	11.2	5.60	0.123	14.9	7.73	3.96	0.147	1.40	1.45	1.50	1.53

Ca (mg/g H_2O) $\times 10^3$				Mg (mg/g H_2O) $\times 10^3$				Zn (mg/g H_2O) $\times 10^3$			
% NaCl				% NaCl				% NaCl			
3.7	1.9	0.97	0.0	3.7	1.9	0.97	0.0	3.7	1.9	0.97	0.0
9.58	8.90	8.77	9.04	81.8	81.4	81.5	79.3	0.384	0.576	0.644	0.691

zinc content, and slightly decreased potassium content. At 70°C, increasing concentration of NaCl increased sodium, chloride, and calcium content; magnesium content remained constant, while zinc content decreased. Heating at 70°C had little or no effect on the sodium, chloride, and potassium content of the extracts, but increased the calcium and magnesium content and decreased the zinc content.

Percentage of total electrolytes and nitrogen present in the aqueous phase. Figs. 1 and 2 show the amount of each electrolyte extracted, expressed as the percentage of the total of each electrolyte present in the meat. The equation

$$\frac{\text{electrolyte content of supernatant (g/g } H_2O) \times \text{total water (g)}}{\text{electrolyte content of meat (g/100 g meat)}} \times 100$$

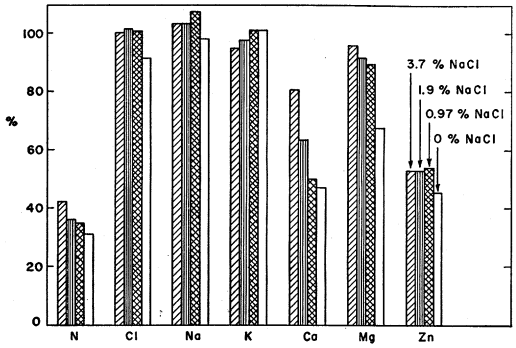

Fig. 1. Average percentages of electrolytes extracted in the aqueous phase of 3°C-treated homogenates.

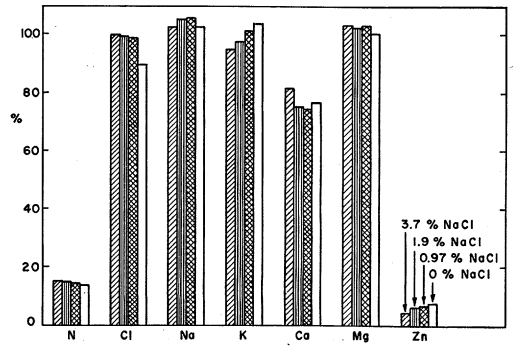

Fig. 2. Average percentages of electrolytes extracted in the aqueous phase of 70°C-treated homogenates.

where total water, g = 200 g + water content of 100 g meat, g was employed in calculating the percentage of electrolyte in the aqueous phase. The assumptions made are that the electrolytes are in equilibrium between the aqueous phase of the supernatant and the aqueous phase retained in the residue. At 3°C, 91.6% of the chloride was soluble in the presence of no added NaCl, while all was soluble in samples containing added NaCl. Sodium and potassium were totally soluble at all concentrations. Increasing NaCl content increased calcium, magnesium, zinc, and nitrogen solubility. Soluble calcium increased from 46.6 to 80.2%; magnesium from 67.2 to 95.8%; zinc from 45.7 to 52.9%; and nitrogen from 31.1 to 42.5%. In homogenates heated at 70°C, sodium, potassium, and magnesium were totally soluble at 0.0% concentration, as they also were on increasing the concentration of NaCl. Chloride was 89.6% soluble in the presence of no added NaCl, and totally soluble at the three levels of added NaCl. Increasing the concentration of NaCl had little effect on the solubility of calcium except for a slight increase at 3.7%. Increasing the concentration of NaCl increased the solubility of nitrogen from 14.1 to 15.7% and decreased the solubility of zinc from 7.7 to 4.5%. Comparison of the amounts of electrolytes soluble in the aqueous fraction of unheated and heated homogenates shows that heating decreased the percentage of soluble nitrogen nearly threefold, had little effect on the solubility of chloride, had no effect on the solubility of sodium and potassium, increased the solubility of calcium at all NaCl levels, produced totally soluble magnesium at all levels, and decreased the solubility of zinc enormously.

Electrolytes and nitrogenous substances in ultracentrifugal supernatant fractions. Table 5 shows the amount of total nitrogen and non-protein nitrogen of a supernatant fraction from a homogenate containing 3.7% added NaCl, before and after ultracentrifugation. As shown in the table, total nitrogen and the non-protein nitrogen contents were practically identical, further showing that a protein-free solution was obtained. Table 6 shows the content of water, nitrogen, chloride, and sodium; and Table 7 shows the content of potassium, calcium, magnesium, and zinc in the supernatant fractions of unheated and heated homogenates before and after ultracentrifugation at 144,000 × G. The content of nitrogen decreased greatly, as expected. Water, chloride, sodium, potassium, calcium, and magnesium decreased to only a small extent. The zinc content decreased greatly. Treatment with NaCl at the 3.7% level, as compared with the 0.0% level, increased the amount of non-protein nitrogen in the supernatant 0.14 mg N/ml H_2O, or 10.9%. The non-protein nitrogen contents of supernatant fractions from treatments with 3.7% NaCl at both 3 and 70°C were similar. Therefore, no fragmentation of protein apparently took place at 70°C. Treatment with 3.7% NaCl at 3°C increased the nitrogen content by 1.59 mg N/ml H_2O; consequently, after correcting for the 0.14 mg N/ml H_2O increase in non-protein nitrogen, 1.45 mg/ml H_2O of salt-soluble protein nitrogen was found to be solubilized by 3.7% NaCl. Comparison of the supernatant fractions from treatment with 3.7% NaCl at 70°C, before and after ultracentrifugation, shows that 0.28 mg N/ml H_2O of protein was sedimented. This amount, representing 18.5% of the salt-soluble protein, was not heat-coagulated at 70°C. Whether

Table 5. Content of nitrogen and non-protein nitrogen (mg/g H_2O) before and after ultracentrifugation.

	Before		After	
	N	NPN	N	NPN
Animal A	4.96	1.97	1.60	1.67
Animal B	5.45	1.64	1.58	1.56

Table 6. Average (3 animals) water, nitrogen, chloride, and sodium content of the supernatants of 3 and 70°C-treated homogenates before and after ultracentrifugation.

H_2O g/g			N mg/g H_2O			Cl mg/g H_2O			Na mg/g H_2O		
3°C	70°C	3°C	3°C	70°C	3°C	3°C	70°C	3°C	3°C	70°C	3°C
% NaCl			% NaCl			% NaCl			% NaCl		
3.7	3.7	0	3.7	3.7	0	3.7	3.7	0	3.7	3.7	0
Before ultracentrifugation											
0.932	0.949	0.972	5.52	1.96	3.93	22.3	22.0	0.136	14.7	14.7	0.159
After ultracentrifugation											
0.956	0.954	0.987	1.70	1.68	1.56	20.9	21.2	0.126	14.3	14.5	0.155

Table 7. Average (3 animals) potassium, calcium, magnesium, and zinc content of the supernatants of 3 and 70°C treated homogenates before and after ultracentrifugation.

K mg/g H_2O			Ca mg/g H_2O ($\times 10^3$)			Mg mg/g H_2O ($\times 10^3$)			Zn mg/g H_2O ($\times 10^3$)		
3°C	70°C	3°C	3°C	70°C	3°C	3°C	70°C	3°C	3°C	70°C	3°C
% NaCl			% NaCl			% NaCl			% NaCl		
3.7	3.7	0	3.7	3.7	0	3.7	3.7	0	3.7	3.7	0
Before ultracentrifugation											
1.52	1.53	1.48	9.23	10.02	5.57	78.2	81.0	61.3	5.74	0.648	4.52
After ultracentrifugation											
1.44	1.46	1.40	8.66	10.14	5.52	65.7	70.0	52.0	1.70	0.653	1.48

Table 8. Average (3 animals) percentage of the nitrogen and electrolyte content of supernatant fractions retained in solution after 24 hr of ultracentrifugation.

N			Cl			Na			K		
3°C	70°C	3°C	3°C	70°C	3°C	3°C	70°C	3°C	3°C	70°C	3°C
% NaCl			% NaCl			% NaCl			% NaCl		
3.7	3.7	0	3.7	3.7	0	3.7	3.7	0	3.7	3.7	0
31.0	85.7	39.6	93.6	96.3	91.9	96.7	98.1	97.4	94.7	95.4	94.8
Ca			Mg			Zn					
93.9	101.2	99.3	84.6	84.6	86.9	32.0	100.9	35.3			

this heat-stable protein represents a portion of the principal salt-soluble proteins or is a specific protein, awaits further study.

Table 8 shows the amount of nitrogen and electrolytes in ultracentrifugally obtained solutions expressed as the percentage of the amount originally present in the supernatant fraction of the homogenates. These figures represent the percentage of each electrolyte in the supernatant that was not bound. Nitrogen content ranged from 31.0 to 85.7%; chloride, from 91.9 to 96.3%; sodium, from 96.7 to 98.1%; potassium, from 94.7 to 95.4%; calcium, from 93.9 to 101.2%; magnesium, from 84.6 to 86.9%; and zinc, from 32.0 to 101.0%. The results show that of the electrolytes determined, only zinc was substantially and strongly associated, or bound, with water- and salt-soluble proteins.

DISCUSSION

The authors have undertaken herein to obtain new information on the role played by the addition of NaCl to meat in meat curing. Investigated were the binding of added sodium and chloride and the distribution of calcium, magnesium, and zinc in increasing concentrations of NaCl and at 3 and 70°C. Also, information was obtained on the amounts of electrolyte bound to water- and salt-soluble protein fractions.

The sodium and chloride results in Table

5 show that little or no binding of sodium or chloride occurred. Such binding should occur if published explanations of the effect of NaCl on the water retention and swelling of meat are valid, namely: a) that electrostatic binding of chloride ion causes increased repulsion of the peptide chains with subsequent absorption of water by capillary condensation (Hamm, 1957); b) that sodium and chloride absorption is an important factor (Sherman, 1962); and c) that sodium ions are preferentially absorbed by the protein imidazole groups (Mahon, 1961). Hamm's (1957) conclusion that preferential chloride ion absorption is important is based on the decreased pH that follows mixing NaCl with meat. Mahon's (1961) conclusion that preferential absorption of sodium ion occurs is based on shifts of titration curves in the acidic region and the additional sodium hydroxide that must be added to attain a given pH value in the presence of NaCl. Both authors measured hydrogen ion concentration potentiometrically and dealt with extremely small ionic shifts. A shift in pH of one unit, as from 5.0 to 6.0, a pH change larger than that achieved experimentally (Wierbicki *et al.*, 1957), results in a total change of 9×10^{-6} millimoles of hydrogen ion per ml, or the equivalent of 207×10^{-6} mg Na/ml and 319×10^{-6} mg Cl/ml, an amount too small to detect by direct analytical techniques. The present findings also do not support the conclusions of Sherman (1962) that sodium and chloride absorption occurs appreciably. Close examination of his results and method of measuring ion absorption raises two possible explanations of the variance of his conclusions with those indicated by the present evidence: 1) that he failed to take into account the water originally present in the meat in performing calculations and 2) that he assumed that ions present in the aqueous phase in the residue were bound to the meat proper, failing to calculate unbound electrolytes in this phase.

In any case, the present results show that the aforementioned explanations by Hamm (1957), Mahon (1961), and Sherman (1962) account only partly for the effects produced by NaCl. At 3°C, NaCl promoted the release of calcium, magnesium, and, to a lesser extent, zinc from the solid phase to the aqueous phase. The ionic movements found were of greater magnitude than the amounts of binding attributed to chloride and sodium ions. By comparing the amount of free ion released by the addition of 3.7% NaCl, as seen by reference to the analyses of ultracentrifuged extracts (Table 9), the amounts of calcium, magnesium, and zinc released were respectively 3.14×10^{-3}, 13.7×10^{-3}, and 0.22×10^{-3} mg/ml. These levels are fortyfold greater than amounts of bound chloride ion and sodium ion.

Heating at 70°C in the presence of no added NaCl promoted the release of all the magnesium and increased by 30% the amount of soluble calcium. A further increase, amounting to 5% of the bound calcium, was produced by adding 3.7% NaCl to the homogenate. The report of Hamm and Deatherage (1960) that free carboxyl groups are destroyed by heating may, in part, explain the release of magnesium and calcium on heating. Heating decreased the amount of zinc in the soluble phase. As shown by the data obtained by ultracentrifugation, some of the free zinc decreased on heating. Work of Edman (1959a,b) showing that zinc is bound to imidazole groups that are unaffected by heating (Hamm and Deatherage, 1960) may explain the continued retention of zinc on the proteins at elevated temperature. The zinc-containing enzymes (Berman, 1961) were presumably precipitated on heating, accounting for part of the decrease of soluble zinc. The report of Gurd and Wilcox (1956), stating that new binding sites may become available on heating, could explain the observed uptake of ionic zinc.

The present results open to question the mode of action attributed to the phosphates when used in conjunction with NaCl. Grau *et al.* (1953a,b) and Hamm (1956, 1957) credit the phosphates with sequestering calcium, magnesium, and zinc ions in raw meat. The results presented, at least in meat held beyond rigor, would tend to minimize the sequestering role of the phosphates, for at 3°C the addition of NaCl increases

by 28.6–33.6% the amount of soluble magnesium and calcium, with but 4 and 20%, respectively, remaining in the insoluble phase. With 47 and 95% of the zinc still in the insoluble phase at 3 and 70°C, respectively, the sequestering role of the phosphates may be limited to the removal of zinc. The effect of zinc in meat has been considered to be similar to that of calcium in that its binding to the structural proteins of meat has been assumed to have an adverse effect on water retention (Hamm, 1958, 1959). However, the direct, highly significant correlation found between water retention and zinc content, in contrast to the inverse relation found between water retention and either calcium or magnesium content (Swift and Berman, 1959), and, now, the contrast in binding between zinc and the other two ions observed in the present study, indicates that zinc differs in an important aspect from the two other ions. In previous studies (Hamm, 1955, 1958), zinc removal has been concomitant with calcium and magnesium removal, which could have obscured its role. The possibility exists that the enhanced hydration observed experimentally and attributed to calcium and magnesium removal may actually have consisted of a composite of effects in which the effect of calcium and magnesium removal could mask the effect of zinc removal, which could possibly have either a negative or positive effect on hydration. The specific effect of zinc on meat hydration remains in doubt.

The validity of treating the supernatant fractions as representative of the entire aqueous phases present in the residues, or throughout the homogenates, was supported by the results of the sodium and chloride analyses. At all concentrations of added NaCl, the analytical results for sodium and chloride were, within experimental error, comparable to the calculated values, i.e., in each case, the water content of the supernatant fraction and the water content of the residue, multiplied by the concentration of NaCl, equaled the amount of NaCl added. Nevertheless, the analyses do not permit the validity of the assumption to be rigorously proven, since the possibility exists that part of the water phase in residues may not have been in equilibrium with the supernatant fractions, in which case the sodium and chloride this water would have contained at equilibrium could actually be bound. It is also recognized that error in determinations of free electrolytes, using the ultracentrifugation method, could result from denaturation of protein or dissociation of electrolyte due to protein sedimentation.

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